C. R. Biologies 338 (2015) 21–28

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Plant biology and pathology/Biologie et pathologie végétales

Integrated effect of microbial antagonist, organic amendment and fungicide in controlling seedling mortality (*Rhizoctonia solani*) and improving yield in pea (*Pisum sativum* L.)

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ARTICLE INFO

Article history:
Received 4 August 2014
Accepted after revision 10 October 2014
Available online 13 November 2014

Keywords:
Trichoderma harzianum
Mustard oilcake
Provax-200
Rhizoctonia solani
Yield

ABSTRACT

The study evaluated the comparative performance of a few microbial antagonists, organic amendments and fungicides and their integration for the management of seedling mortality (*Rhizoctonia solani* Kühn) and yield improvement in pea (*Pisum sativum* L.). Before setting the experiment in field microplots, a series of *in vitro* and *in vivo* experiments were conducted to select a virulent isolate of *R. solani*, an effective antagonistic isolate of *Trichoderma harzianum*, a fungitoxic organic amendment and an appropriate fungicide. A greenhouse pathogenicity test compared differences in seedling mortality in pea inoculated by four isolates of *R. solani* and identified the isolate RS10 as the most virulent one. Among the 20 isolates screened in dual culture assay on PDA, *T. harzianum* isolate T-3 was found to show the highest (77.22%) inhibition of the radial growth of *R. solani*. A complete inhibition (100.00%) of colony growth of *R. solani* was observed when fungicide Bavistin 50 WP and Provax-200 at the rate of 100 and 250 ppm, respectively, were used, while Provax-200 was found to be highly compatible with *T. harzianum*. Mustard oilcake gave maximum inhibition (60.28%) of the radial growth of *R. solani* at all ratios, followed by sesame oilcake and tea waste. Integration of soil treatment with *T. harzianum* isolate T-3 and mustard oilcake and seed treatment with Provax-200 appeared to be significantly superior in reducing seedling mortality and improving seed yield in pea in comparison to any single or dual application of them in the experimental field. The research results will help growers develop integrated disease management strategies for the control of *Rhizoctonia* disease in pea. The research results show the need for an integrating selective microbial antagonist, organic amendment and fungicide to achieve appropriate management of seedling mortality (*R. solani*) and increase of seed yield in pea.

1. Introduction

Pea (*Pisum sativum* L.) is a popular leguminous vegetable in Bangladesh. It is grown mainly for green pods and immature seeds, which are consumed as vegetables. Pea has also a great agronomic value. In crop rotation, it helps in improving soil fertility and the yield of succeeding crops. Diseases are one of the main constraints

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http://dx.doi.org/10.1016/j.crvi.2014.10.003
1631-0691/

for successful pea cultivation. Eleven diseases of pea are reported in Bangladesh, causing about 30–40% yield loss annually [1]. *Rhizoctonia solani* causing seedling mortality and root rot is one of the major diseases. The fungus most often infects the hypocotyl, epicotyl and seed of pea [2]. The management of *R. solani* is difficult due to its soil-borne nature. The fungus is present in most of the soil. Once it is established in the field, it remains indefinitely there [3]. Dry sclerotia of the pathogen are reported to survive up to 6 years when stored at room temperature. Several fungicides, effective against *R. solani*, can be used for soil treatment as well as for treating material to be planted [4]. However, persistent and injudicious use of chemicals is usually associated with some familiar problems such as toxic effects on non-target organisms, development of fungicide-resistant pathogenic strains and undesirable changes on environment. *Trichoderma harzianum* is one efficient biological control agent and plant growth promoter that is successfully used to suppress *R. solani* and improve yield in economic crops [5–8]. Many reports also indicate that amending soil with organic amendments such as oil seed cake and plant residues significantly reduces soil-borne pathogens including *R. solani* and increases crop productivity [9,10]. However, the most economical way of controlling the disease is the use of a resistant variety, but none among the existing ones was reported to be resistant to the pathogen. Also the strong pathogen inocula in the soil limit the performance of any resistant variety.

Because of the above considerations and unattainability of real varietal resistance against rhizoctoniasis in pea, research directed towards reducing the soil-borne inoculum of *R. solani* should be taken into serious consideration. However, it is very expensive and not sufficient to escape the pathogen in infested field soil with a single control measure/tactic [11]. Thus, in the case of *Rhizoctonia* diseases, since chemical control was not successful in many situations and host resistance is rare, integrated control has often been pursued as the only means of achieving reliable and economic disease reduction. Integrated control of *Rhizoctonia* diseases employs combinations of biological, chemical and cultural approaches. Combined strategies are often additive and sometimes synergistic, leading to more effective and reliable disease control. Until now, many integrated systems have been researched and some are being implemented at the farm level. In each occasion, the most critical step to developing an effective integrated disease management system was the identification of the best and compatible mix of biological, chemical and cultural tactics for a given pathogen. Unfortunately, the interest in such comprehensive study and its progress are completely lacking in Bangladesh, hampering the development of integrated management systems in this discipline. In the present work, we conducted experiments to identify the best individual treatment of antagonists, organic amendments and fungicides for their efficacy in *R. solani* inhibition and investigated the individual and combined effect of them in the experimental field on seedling mortality disease and yield in pea.

2. Materials and methods

2.1. Microorganisms and plant materials

All the isolates (T-1, T-2, T-3, T-4, T-5, T-6, T-7, T-8, T-9, T-10, T-11, T-12, T-13, T-14, T-15, T-16, T-17, T-18, T-19 and T-20) of *T. harzianum* were collected from the stock cultures of the Plant Pathology Laboratory of the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Four isolates of the test pathogen *R. solani* were isolated from pea, cotton and bush bean. "IPSA motorshuti-3" variety of pea was used as the test plant.

2.2. Isolation and characterization of R. solani

Infected root, leaf, petiole, and stem tissues of pea, cotton and bush bean were collected from the field laboratory of BSMRAU and washed in distilled water to remove sand and soil particles. The specimens were cut into small pieces (5 mm) along with healthy and dead tissue and surface sterilized with 0.1% $NaOCl_2$ for 2 min and rinsed in sterilized water for three times. The plant pieces were blotted dry to remove excess water and plated on water agar. The characteristic colony of *R. solani* was transferred to fresh acidified PDA (pH 4.5). These isolates were purified by selecting a single hyphal tip (less than 1.5 mm) of each isolate growing on PDA to obtain pure cultures. All isolates were maintained in the short term on PDA slants and on colonized wheat seeds for longer-term storage. Each isolate was arbitrarily named with capital letter codes and a serial number (Table 1). Selected isolates were inoculated onto four replicate PDA plates using 5-mm-diameter mycelial disks taken from the margin of a 3-day-old PDA culture, and the plates were sealed with parafilm strips and incubated in airtight plastic containers at room temperatures (25 ± 2 °C). After 60 h of incubation, observation on several cultural characteristics such as colony colour, mycelial

Table 1
Cultural characteristics and pathogenicity of the isolates of *Rhizoctonia solani*.

R. solani isolates	Source Crop	Colony colour	Mycelial density	Zonation	No. of sclerotia/plate	% Pre-emergence mortality	% Post-emergence mortality	% Total mortality
RS1	Cotton	Brown	IV	2	11.58	33.33	33.33	66.67
RS3	Pea	Brown	IV	2	14.46	90.47	4.76	95.23
RS7	Bush bean	Brown	IV	2	10.34	85.71	9.52	95.23
RS10	Pea	Brown	IV	3	17.34	100.00	0.00	100.00
Control	–	–	–	–	–	0.00	0.00	0.00